

IDENTIFICATION AND CHARACTERISATION OF QUANTITATIVE TRAIT  
LOCI LINKED WITH TOLERANCE TO SALINE-ALKALINE STRESS AT  
GERMINATION STAGE IN *INDICA* RICE (*Oryza sativa* L.)

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Specially dedicated to my father Umar Hayat Khan Niazi  
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## ABSTRACT

As the direct seeding of rice is getting popular, the farmers have shifted from traditional transplantation method to direct seeding method. The problem with this method however is the direct exposure of seeds to abiotic stresses that affect crop establishment. Studies have not been conducted so far to improve the germination tolerance in saline-alkaline conditions. Therefore, the present study was conducted to perform the genomic analysis of tolerance to saline-alkaline stress at germination stage in rice. Parental varieties *Pokkali* and *Pak Basmati* were selected out of six rice varieties on the basis of their performance in saline-alkaline conditions. A mapping population (F<sub>2:3</sub>) was constructed using *Pokkali* as tolerant and *Pak Basmati* as sensitive parent. Germination parameters such as final germination percentage (FGP), germination value (GV), germination energy (GE), germination velocity (GVe), speed of germination (SG), peak value (PV), germination capacity (GC), germination index (GI) and mean germination time (MGT) while growth parameters like germination vigour index (GVI), shoot length (SL), root length (RL) and total dry biomass (DBM) were recorded. Parental varieties were optimised for saline-alkaline stress limits. Phenotyping of F<sub>3</sub> progenies was conducted with the saline-alkaline limits (pH8/15 dS.m<sup>-1</sup>) optimised in parental varieties. Correlation studies show that germination and growth parameters are positively correlated. It was also found that growth parameters were affected more than germination parameters. The genotypic data of 129 F<sub>2</sub> plants for 84 polymorphic markers was used to construct molecular linkage map, with an average interval size of 7.63cM with four gaps of  $\leq$  40cM and the total length of 3435.5cM. Quantitative Trait Loci (QTL) Cartographer was used for genomic analysis using three mapping techniques i.e. simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM). Thirty-three QTLs (17 major and 16 minor) were identified using SIM. Forty QTLs (14 major and 26 minor) were identified by CIM. Sixty QTLs were identified using MIM technique. The parental varieties used in this study are potential candidates for abiotic stress studies. These findings would be beneficial in rice breeding programs to develop tolerant cultivars for the saline-alkaline environment through marker assisted selection.

## ABSTRAK

Memandangkan pembenihan tabur terus padi semakin popular, petani telah beralih dari kaedah menanam secara tradisional ke kaedah tabur terus. Masalah dengan kaedah ini bagaimanapun adalah pendedahan benih secara terus kepada tekanan abiotik telah menjejaskan pertumbuhan tanaman. Sehingga kini kajian untuk meningkatkan toleransi percambahan dalam keadaan masin-alkali tidak pernah dijalankan. Oleh itu, kajian ini dijalankan untuk melaksanakan analisis genom toleransi kepada tekanan masin-alkali pada peringkat percambahan dalam padi. Jenis ibu bapa *Pokkali* dan *Pak Basmati* telah dipilih daripada enam jenis padi berdasarkan prestasi mereka dalam keadaan masin-alkali. Pemetaan Populasi ( $F_{2:3}$ ) telah dibina menggunakan *Pokkali* sebagai toleran dan *Pak Basmati* sebagai induk yang sensitif. Percambahan parameter seperti peratus percambahan akhir (FGP), nilai percambahan (GV), percambahan tenaga (GE), halaju percambahan (GVe), kelajuan percambahan (SG), nilai puncak (PV), kapasiti percambahan (CA), indeks percambahan (GI) dan min masa percambahan (MGT) manakala parameter pertumbuhan seperti indeks percambahan tenaga (GVI), tempoh bertunas (SL), tempoh asal (RL) dan jumlah biojisim kering (DBM) telah direkodkan. Saluran induk telah dioptimumkan untuk had tekanan masin-alkali. Penelitian lapangan (*Phenotyping*) progeni  $F_3$  telah dijalankan dengan had masin-alkali ( $\text{pH}8 / 15 \text{ dS.m}^{-1}$ ) yang dioptimumkan dalam jenis ibu bapa. Kajian korelasi menunjukkan bahawa percambahan dan pertumbuhan parameter berkorelasi secara positif. Ia juga mendapati bahawa parameter pertumbuhan lebih terjejas daripada parameter percambahan. 129 data genotip tumbuhan  $F_2$  untuk 84 penanda polimorf telah digunakan untuk membina peta rangkaian molekul, dengan saiz selang purata sebanyak 7.63cM dengan empat jurang sebanyak  $\leq 40\text{cm}$  dan jumlah panjang sebanyak 3435.5cM. Ciri Kuantitatif Loci (QTL) telah digunakan untuk analisis genom menggunakan tiga teknik pemetaan iaitu Pemetaan Selang Mudah (SIM), Pemetaan Selang Komposit (CIM) dan Pemetaan Selang Berganda (MIM). Tiga puluh tiga QTLs (17 utama dan 16 kecil) telah dikenal pasti menggunakan SIM. Empat puluh QTLs (14 utama dan 26 kecil) telah dikenal pasti oleh CIM. Enam puluh QTLs telah dikenal pasti menggunakan teknik MIM. Jenis ibu bapa yang digunakan dalam kajian ini adalah calon berpotensi untuk kajian tekanan abiotik. Hasil kajian ini memberi manfaat dalam program pembenihan padi untuk membangunkan kultivar toleran untuk persekitaran masin-alkali melalui bantuan penanda pilihan.

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## LIST OF ABBREVIATIONS

$\mu\text{mol.m}^{-2}.\text{s}^{-1}$	-	Micromole per meter square. per second
1N	-	One Normal
AFLP	-	Amplified Fragment Length Polymorphism
AFLPs	-	Amplified Fragment Length Polymorphisms
ANOVA	-	Analysis of Variance
APS	-	Ammonium persulfate
BC	-	Backcross
BC1	-	First Backcross Generation
BC <sub>1</sub> F <sub>2</sub>	-	Second Backcross Generation after selfing
bp	-	Base pair
C	-	Codominant
Ca <sup>2+</sup>	-	Calcium ion
Chr	-	Chromosome
Chr No	-	Chromosome Number
CIM	-	Composite Interval Mapping
Cl <sup>-</sup>	-	Chloride anion
cm	-	Centimeter
cM	-	centiMorgan
CO <sub>3</sub> <sup>2-</sup>	-	Carbonate ion
CTAB	-	Cetyl Trimethyl Ammonium Bromide
D	-	Dominant
DBM	-	Dry Bio Mass
df	-	Degree of Freedom
DH	-	Double Haploids
DH	-	Double haploid

DHLs	-	Double Haploid Lines
DNA	-	Deoxyribonucleic Acid
dS.m <sup>-1</sup>	-	deciSiemens per meter
EC	-	Electric Conductivity
EC <sub>e</sub>	-	Electrical Conductivity
EDTA	-	Ethylenediaminetetraacetic acid
E-QTLs	-	Epistatic Quantitative Trait Loci
ESP	-	Exchangeable Sodium Percentage
F <sub>1</sub>	-	First filial generation
F <sub>2</sub>	-	Second filial generation
F <sub>2:3</sub>	-	Second filial generation advanced to third filial generation
F <sub>2:4</sub>	-	Second filial generation advanced to fourth filial generation
F <sub>3</sub>	-	Third filial generation
FAO	-	Food and Agriculture Organization
FGP	-	Final Germination Percentage
Fwd	-	Forward
g	-	Gram
GC	-	Germination Capacity
GE	-	Germination Energy
GI	-	Germination Index
GV	-	Germination Value
GVe	-	Germination Velocity
GVI	-	Germination Vigour Index
ha	-	Hectare
HCl	-	Hydrogen Chloride
HCO <sub>3</sub> <sup>-</sup>	-	Bicarbonate ion
IM	-	Interval Mapping
IRRI	-	International Rice Research Institute
IRRI	-	International Rice Research Institute
K <sup>+</sup>	-	Potassium ion
kb	-	kilo base

LED	-	Light emitting diode
LOD	-	Logarithm of Odd
LRT	-	Likelihood Ratio Test
MapQTL	-	Mapping Quantitative Trait Loci
MAS	-	Marker Assisted Selection
Mg <sup>2+</sup>	-	Magnesium ion
MgCl <sub>2</sub>	-	Magnesium Chloride
mgkg <sup>-1</sup>	-	milligram per kilogram
MGT	-	Mean Germination Time
MIM	-	Multiple Interval Mapping
min	-	Minute
mM	-	milliMolar
mmol	-	Millimolar
MPa	-	Mega Pascal
M-QTLs	-	Main effect Quantitative Trait Loci
Na <sup>+</sup>	-	Sodium ion
NaCl	-	Sodium chloride
NaCl	-	Sodium Chloride
NaOCl	-	Sodium Hypochlorite
ngμl <sup>-1</sup>	-	Nano gram per microliters
NO <sub>3</sub> <sup>-</sup>	-	Nitrate ion
OD	-	Optical Density
<i>p</i>	-	Significance Level
P <sub>1</sub>	-	Parent 1 used in crossing
P <sub>2</sub>	-	Parent 2 used in crossing
PAGE	-	Poly Acrylamide Gel Electrophoresis
PCR	-	Polymerase Chain Reaction
pH	-	Power of hydrogen
PIC	-	Polymorphism information content
PV	-	Peak Value
QTL	-	Quantitative Trait Loci
<i>r</i>	-	Pearson correlation
R <sup>2</sup>	-	Phenotypic Variation



Rev	-	Reverse
RFLP	-	Restriction Fragment Length Polymorphism
RILs	-	Recombinant Inbred Lines
RL	-	Root Length
RM	-	Rice Marker
sec	-	Second
SG	-	Speed of Germination
SIM	-	Simple Interval Mapping
SL	-	Shoot Length
SNPs	-	Single Nucleotide Polymorphisms
SO <sub>4</sub> <sup>2-</sup>	-	Sulfate ion
SSRs	-	Simple Sequence Repeats
TBE	-	Tris Boric acid EDTA buffer
TE	-	Tris EDTA buffer
TEMED	-	Tetramethylethylenediamine
Tris-HCl	-	Tris hydrochloride
V or v	-	Version
WinQTLCart	-	Windows Quantitative Trait Loci Cartographer
$\chi^2$	-	Chi Square
$\mu\text{L}$	-	Microliters

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## CHAPTER 1

### INTRODUCTION

#### 1.1 Background of Study

Rice (*Oryza sativa* L.) is one of the most important cereal crops of the world. It is essential source of staple food for more than 2.7 billion people all around the globe, most of them living in developing countries (FAO, 2016). It is cultivated on one-tenth of the arable land of the planet earth. By the year 2025, a total increase of 21% would be needed to meet the food requirements compared to the needs in year 2000 (Bhowmik *et al.*, 2007). Second only to wheat, rice production contributes to almost 20% of the total cereal crop production of the world (Acquaah, 2007). Rice is second biggest crop produced all over the world with a total of 740.95 million tons produced from 163.1 million hectares at an average of 4.54 tons of rice per hectare (FAO, 2010). In addition to its importance as one of the biggest sources of food, rice is rich in genetic diversity as there are thousands of varieties and accessions all over the world (Ammar *et al.*, 2007). There are two main methods being practised for rice cultivation. Traditional transplanting system (TPR), an important plant establishment technique where pre-germinated seedlings are transferred to the fields (Farooq *et al.*, 2007). The seedlings are provided ideal growth conditions before being transplanted. The second method is direct seeding of rice (DSR) which entails broadcasting the seeds directly through hands or machines (Farooq *et al.*, 2011). Both these methods have their own pros and cons.

TPR requires labour and time, and it is economically expensive as well. Handling the pre-germinated seeds is difficult (Farooq *et al.*, 2007). While DSR has a major drawback that seeds are directly exposed to abiotic stresses (Farooq *et al.*,

2011). Cultivation areas are shrinking because of rapid urbanization and industrialization (Jiang and Li, 2016). Therefore, utilisation of less productive or saline-sodic marginal soils would be a suitable option to bring under cultivation by improving the crops tolerance to saline-alkalinity. Ever increasing population demands higher amounts of rice and other food crops. This can be achieved by producing high yielding varieties, by expanding the total area presently under cultivation or by following the latest management practices to increase the overall rice production. However increased population and natural disasters causing a direct threat to the food security (Gardi *et al.*, 2015; Gardner, 1996; Nath *et al.*, 2015) hence expanding the cultivated areas is becoming hard. There are variety of abiotic and biotic stresses affecting the food production adversely, in general and rice production in particular. Only in Asia these stresses cause a total of 23% of production decrease compared to its full potential (Hossain, 1997). Salinity is second biggest abiotic stress condition after drought, in rice cultivating areas. Thirty percent of total rice grown area all over the world is severely affected by high salinity levels. These levels adversely affect the normal growth of rice plants.

A total of  $1.5 \times 10^9$  ha land area is cultivated all over the world out of which 23% area is saline and another 37% are sodic in nature (Shi and Wang, 2005). The cations present in the saline and sodic soils are  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$  and  $\text{K}^+$  and anions  $\text{Cl}^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{HCO}_3^-$ ,  $\text{CO}_3^{2-}$  and  $\text{NO}_3^-$  are present. Studies have shown that alkali stress results mainly from certain levels of sodium bicarbonate and sodium carbonate (Shi and Yin, 1993). Quite a lot of literature is available on salt stress (Munns and Tester, 2008) while a little attention was paid to the saline-alkaline stress. Saline-sodic soils occur within the boundaries of 75 countries and the area under saline-alkalinity is gradually increasing (Qadir *et al.*, 2007). Sodic and saline-sodic soils are grouped together because of sharing same characteristics and the management practices (Qadir *et al.*, 2007). Rice seeds are affected by these abiotic stresses when broadcasted directly through DSR. It was observed that alkalinity was more damaging than salinity at germination stage (Guo *et al.*, 2010). Plants accumulate more  $\text{Na}^+$  at the expense of  $\text{K}^+$ . They also start accumulating soluble osmolytes which are compounds affecting osmosis. Severe inhibition in wheat root and shoot under saline-alkaline conditions has also been reported (Guo *et al.*, 2010). Grain

security is becoming a major concern and urbanization is one of the factors behind food crisis. Effective land use planning is critical for food security but soil erosion, land degradation and climatic changes are causing a rapid increase in marginal lands (Kang *et al.*, 2013). Scherr, (1999) has emphasized on the reduced annual yields and role of soil quality in overall crop production and also emphasized on the need to improve the soil quality. We have marginal soils i.e. the soils with problems but we do not have the varieties that could grow there because we do not have much knowledge or data about tolerance to abiotic stress. Because of these factors, the crops are becoming low yielding. Already cultivated area is shrinking because of growing salinity, sodicity and drought (Valipour, 2014).

A prudent measure to address the salinity and sodicity issue is to develop tolerant varieties. The recent developments in the field of quantitative genetics have greatly influenced the study of complex quantitative traits and this has made it possible to dissect the complex quantitative traits or polygenes for certain traits to Mendelian factors. Identification of loci controlling genetic variations in segregating populations has become possible with the use of molecular markers and QTL analysis technology. Characterisation of these loci according to their position on genetic map, their mode of action, phenotypic and pleiotropic effects and epistatic interactions with other QTLs have also become possible (Dufey *et al.*, 2015; Ogawa *et al.*, 2016; Zhao *et al.*, 2016). Several studies have been conducted for salinity tolerance in rice at different stages (Heenan *et al.*, 1988b; Kumar *et al.*, 2015; Rahman *et al.*, 2016), however, 37% of the total cultivated land is sodic as well, and it is urgently needed to see and dissect combined effects of salinity and sodicity. Rice is considered as the moderately salinity sensitive cereal crop plant which can tolerate saline levels of 4-8 dS.m<sup>-1</sup> (Sairam and Tyagi, 2004).

The genetic variation that a rice plant possesses makes it suitable candidate for quantitative trait studies. This variation helps not only in understanding the mechanisms behind biotic and abiotic stress studies but also to develop new varieties with improved abiotic and biotic stress tolerance. There have been efforts to develop salt tolerant rice varieties (Flowers, 2004; Khan *et al.*, 2016; Lutts *et al.*, 1995). Genetic variability for salinity tolerance has been reported among a large number of

economically important crops including rice (Flowers, 2004). This genetic information about salt tolerance is essential for developing salt tolerant rice cultivars. This information can later be used in marker assisted selection (MAS) and plant breeding studies to exploit the identified genomic regions known as quantitative trait loci (QTLs).

Saline soils have high pH levels (alkaline) and affect the uptake of nutrients and limit the germination. Studies to improve tolerance to saline-alkaline stress in rice at germination stage has not been conducted yet. QTL identification is another way to speed up breeding tolerant rice varieties through marker assisted selection. Present study focused on genetic dissection of saline-alkaline tolerance mechanisms.

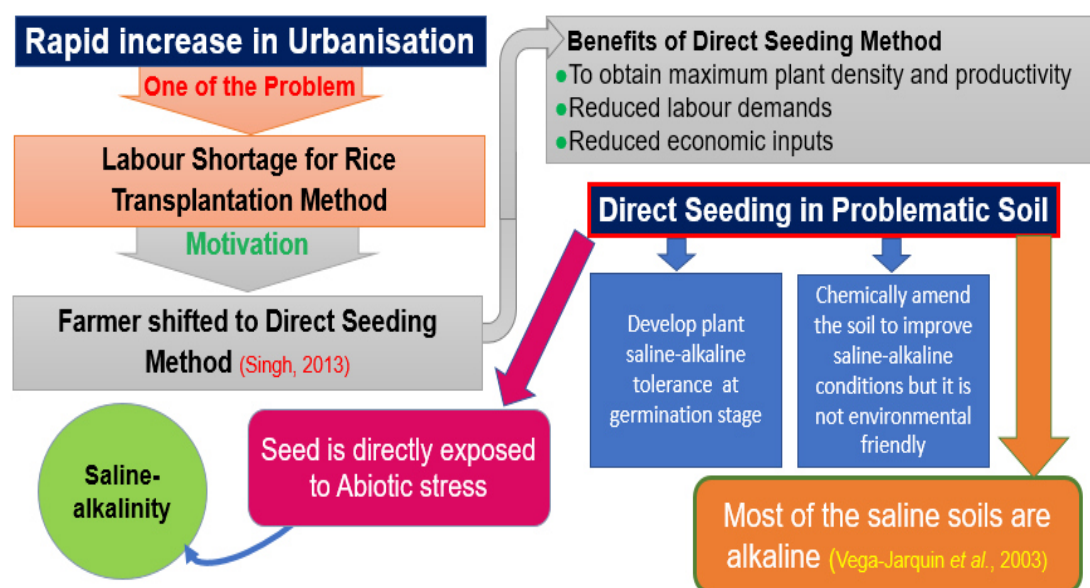
Farmers follow two methods for rice cultivation; transplantation and direct seeding of rice (Singh, 2013). Transplantation is done both manually and mechanically where seedlings are provided with the ideal growth conditions and are transferred to the field when seedlings are 3 to 4 weeks old (Farooq *et al.*, 2007) depending on type of rice varieties. Therefore, it is considered expensive because of labour (Coelli *et al.*, 2002) and water use (Bhushan *et al.*, 2007). Reduced tillage and direct seeding on flat land and raised beds can result in significant water savings (Gupta *et al.*, 2003). Thus, direct seeding is an efficient and economical rice planting option. Plants grown by direct seeding get mature 7-10 days earlier than those transplanted (Farooq *et al.*, 2011).

## **1.2 Problem Statement**

Rapid urbanization and industrialization resulted in labour shortage for the rice transplantation from nurseries to the irrigated paddy fields. It motivated the farmers to shift from transplantation method to direct seeding of rice. Direct seeding of rice is also suitable for the farmers because they can obtain maximum plant density and productivity with lesser labours and low economic inputs. Direct seeded rice reduces the crop water requirements, soil organic-matter turnover, nutrient

relations, carbon sequestrating, weed biota and greenhouse-gas emissions (Farooq *et al.*, 2011). Therefore, direct seeding is more popular among farming community. Majority of the saline soils are alkaline in nature (Vega-Jarquín *et al.*, 2003). When direct seeding method is used where the soils are affected with salinity or alkalinity the seed is exposed directly to the stresses. These abiotic stresses inhibit the seed germination adversely affecting the crop establishment (Vinocur and Altman, 2005). There are two ways to address this problem. One of them is to do the chemical amendments to improve saline-alkaline soil which is not durable and often not environmental friendly (Hai *et al.*, 2010). The second way is to develop the plant saline-alkaline tolerance genetically at germination stage.

Rice is reported sensitive to salinity at different growth stages (Al-Karaki, 2001). The genetic studies revealed the quantitative trait loci (QTLs) linked with tolerance at these growth stages (Koyama *et al.*, 2001; Cheng *et al.*, 2008). However, direct seeding is a new rice culture method and the studies regarding tolerance to saline-alkaline stress at germination stage in rice has not been conducted yet. Present study as shown in Figure 1.1 focused on identification of QTLs linked with tolerance to saline-alkaline stress using  $F_{2:3}$  segregating population.



**Figure 1.1** Illustration of problem statement

### 1.3 Research Objectives

- i. To develop a mapping population to dissect the genetic background for tolerance to saline-alkaline stress at germination stage in *Indica* rice.
- ii. To investigate the tolerance potential in F<sub>3</sub> progenies against the stress limits optimized in parental lines.
- iii. To construct a microsatellite molecular linkage map based on F<sub>2</sub> microsatellite data using MapMaker.
- iv. To identify the microsatellite loci associated with seed germination tolerance to saline-alkalinity based on molecular linkage map and F<sub>3</sub> progenies stress tolerance data using QTL cartographer.

### 1.4 Research Scope

A preliminary study was conducted to select tolerant and sensitive rice varieties under saline-alkaline conditions to develop mapping population. *Pokkali* and *Pak Basmati* were selected as tolerant and sensitive varieties, respectively. These varieties were crossed and F<sub>2</sub> population was developed after confirming the pedigree of F<sub>1</sub> seedlings. F<sub>2</sub> (129) population was used to get marker data by using 84 microsatellite markers. Marker data was used to construct molecular linkage map by MapMaker. F<sub>3</sub> progenies (129) were phenotyped under an optimised stress level. Potential QTLs were identified linked to germination traits under saline-alkaline conditions by three methods simple interval mapping (SIM), composite interval mapping (CIM) and multiple interval mapping (MIM) using QTL cartographer.



## **1.5 Significance of Research**

This study was carried out in order to identify the QTLs responsible for tolerance to saline-alkaline stress at germination stage in rice. The QTLs identified in this study would be used for molecular screening of breeding population i.e. marker assisted breeding. Therefore, it would speed up the rice breeding program to induce tolerance against saline-alkaline stress to develop new rice varieties for such affected soils where direct seeding rice culture is dominated.

## REFERENCES

- Abrol, I. P., Yadav, J. S. P., and Massuod, F. I. (1988). Salt affected soils and their management. Soil Resources, Management and Conservation Service, FAO Land and Water Development Division. *FAO Soils Bulletin*, 39.
- Abu-Sharar, T. M. (1988). Response of four barley cultivars to salinity/sodicity stress. *Dirasat (Agriculture Science)*, 15, 56-65.
- Acquaah, G. (2007). Principles of plant breeding and genetics. *Malden, MA USA: Blackwell* 109-120.
- Acquaah, G. (2009). *Principles of plant genetics and breeding*: Blackwell Publishing Ltd., 350 Main Street, Malden, MA, USA 540-569.
- Ahmadi, J., and Fotokian, M. H. (2011). Identification and mapping of quantitative trait loci associated with salinity tolerance in rice (*Oryza sativa*) using SSR markers. *Iranian Journal of Biotechnology*, 9(1), 21-30.
- Ahn, S. N. (2016). *High-Density Mapping Reveals a Linkage of Quantitative Trait Loci for Grain-Weight and Spikelet Number in Rice*. Paper presented at the Plant and Animal Genome XXIV Conference 715-728.
- Akbar, M., Khush, G. S., and Hillerislambers, D. (1985). *Genetics of salt tolerance in rice*. Paper presented at the Proceeding Of International Rice Genetics Symposium IRRI. 399-409.
- Akbar, M., and Ponnampereuma, F. N. (1982). Saline soils of South and Southeast Asia as potential rice lands. *Rice Research Strategies For The Future. IRRI*, 265-281.
- Akhtar, S., Bhat, M. A., Wani, S. A., Bhat, K. A., Chalkoo, S., Mir, M. R., and Wani, S. A. (2010). Marker assisted selection in rice. *Journal of Phytology*, 2(10), 66-81.
- Al-Karaki, G. N. (2001). Germination, sodium, and potassium concentrations of barley seeds as influenced by salinity. *Journal of Plant Nutrition*, 24(3), 511-522.

- Alam, A. Z., Khan, S., and Javed, M. A. (2016). *Response of Germination Attributes to Salinity Toxicity in Indica Rice (Oryza sativa L.)*. Paper presented at the Proceedings of 4th International Science Postgraduate Conference 2016 (ISPC2016).
- Alam, R., Sazzadur, R. M., Seraj, Z. I., Thomson, M. J., Ismail, A. M., Tumimbang, R. E., and Gregorio, G. B. (2011). Investigation of seedling-stage salinity tolerance QTLs using backcross lines derived from *Oryza sativa* L. cv. *Pokkali*. *Plant Breeding*, 130(4), 430-437.
- Alhasnawi, A. N., Kadhimi, A. A., Isahak, A., Mohamad, A., Yusoff, W. M. W., and Zain, C. R. C. M. (2015). Exogenous application of ascorbic acid ameliorates detrimental effects of salt stress in rice (MRQ74 and MR269) seedlings. *Asian Journal of Crop Science*, 7(3), 186-196.
- Ali, S., Gautam, R., Mahajan, R., Krishnamurthy, S., Sharma, S., Singh, R., and Ismail, A. (2013). Stress indices and selectable traits in SALTOL QTL introgressed rice genotypes for reproductive stage tolerance to sodicity and salinity stresses. *Field Crops Research*, 154(1), 65-73.
- Almansouri, M., Kinet, J.-M., and Lutts, S. (2001). Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). *Plant and Soil*, 231(2), 243-254.
- Ammar, M. H. M., Singh, R. K., Singh, A. K., Mohapatra, T., Sharma, T. R., and Singh, N. K. (2007). *Mapping QTLs for salinity tolerance at seedling stage in rice (Oryza sativa L.)*. Paper presented at the African Crop Science Conference Proceedings, 617-620.
- Anbumalarmathi, J., and Mehta, P. (2013). Effect of salt stress on germination of *Indica* rice varieties. *European Journal of Biological Sciences*, 6(1), 1-6.
- Andaya, V., and Mackill, D. (2003). Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. *Journal of Experimental Botany*, 54(392), 2579-2585.
- Anderson, J. A., Stack, R. W., Liu, S., Waldron, B. L., Fjeld, A. D., Coyne, C., Moreno-Sevilla, B., Fetch, J. M., Song, Q. J., and Cregan, P. B. (2001). DNA markers for Fusarium head blight resistance QTLs in two wheat populations. *Theoretical and Applied Genetics*, 102(8), 1164-1168.
- Angaji, S. A. (2009). QTL mapping: A few key points. *International Journal of Applied Research in Natural Products*, 2(2), 1-3.

- Asch, F., and Wopereis, M. C. (2001). Responses of field-grown irrigated rice cultivars to varying levels of floodwater salinity in a semi-arid environment. *Field Crops Research*, 70(2), 127-137.
- Ashraf, C., and Abu-Shakra, S. (1978). Wheat seed germination under low temperature and moisture stress. *Agronomy Journal*, 70(1), 135-139.
- Ashraf, M. (2004). Some important physiological selection criteria for salt tolerance in plants. *Flora-Morphology, Distribution, Functional Ecology of Plants*, 199(5), 361-376.
- Balkunde, S., Le, H. L., Lee, H. S., Kim, D. M., Kang, J. W., and Ahn, S. N. (2013). Fine mapping of a QTL for the number of spikelets per panicle by using near-isogenic lines derived from an interspecific cross between *Oryza sativa* and *Oryza minuta*. *Plant Breeding*, 132(1), 70-76.
- Bandillo, N., Raghavan, C., Muyco, P. A., Sevilla, M. A. L., Lobina, I. T., Dilla-Ermita, C. J., Tung, C. W., McCouch, S., Thomson, M., and Mauleon, R. (2013). Multi-Parent Advanced Generation Inter-Cross (Magic) Populations in Rice: Progress and Potential for Genetics Research and Breeding. *Rice*, 6(1), 1.
- Bennett, M. D., and Smith, J. B. (1991). Nuclear DNA amounts in angiosperms. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 334(1271), 309-345.
- Bernstein, L. (1975). Effects of salinity and sodicity on plant growth. *Annual Review of Phytopathology*, 13(1), 295-312.
- Bhowmik, S. K., Islam, M. M., Emon, R. M., Begum, S. N., Siddika, A., and Sultana, S. (2007). Identification of salt tolerant rice cultivars via phenotypic and marker-assisted procedures. *Pakistan Journal of Biological Sciences: PJBS*, 10(24), 4449-4454.
- Bhullar, N. K., and Gruissem, W. (2013). Nutritional enhancement of rice for human health: the contribution of biotechnology. *Biotechnology Advances*, 31(1), 50-57.
- Bonilla, P., Mackell, D., Deal, K., and Gregorio, G. (2002). RFLP and SSLP mapping of salinity tolerance genes in chromosome 1 of rice (*Oryza sativa* L.) using recombinant inbred lines. *Philippine Agricultural Scientist (Philippines)*, 65(1), 68-76.

- Boyer, J. S. (1982). Plant productivity and environment. *Science*, 218(4571), 443-448.
- Bray, E. A., Bailey-Serres, J., and Weretilnyk, E. (2000). Responses to abiotic stresses. *Biochemistry and Molecular Biology of Plants*, 1158-1249.
- Buffart, T. E., Tijssen, M., Krugers, T., Carvalho, B., Smeets, S. J., Brakenhoff, R. H., Grabsch, H., Meijer, G. A., Sadowski, H. B., and Ylstra, B. (2007). DNA quality assessment for array CGH by isothermal whole genome amplification. *Analytical Cellular Pathology*, 29(4), 351-359.
- Cai, H.-Y., Diao, S., He, Y.-G., Zhang, L.-P., Liu, S.-J., Zhu, Y.-G., and Zhang, Z.-H. (2012). Genetic and physical mapping of qHY-8, a pleiotropic QTL for heading date and yield-related traits in rice. *Euphytica*, 184(1), 109-118.
- Cheema, J., Ellis, T. H. N., and Dicks, J. (2010). THREaD Mapper Studio: a novel, visual web server for the estimation of genetic linkage maps. *Nucleic Acids Research*, 38(suppl 2), W188-W193.
- Chen, X., Temnykh, S., Xu, Y., Cho, Y., and McCouch, S. (1997). Development of a microsatellite framework map providing genome-wide coverage in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 95(4), 553-567.
- Cheng, H. T., Jiang, H., Da-Wei, X. U. E., Long-Biao, G. U. O., Da-Li, Z., Zhang, G. H., and Qian, Q. (2008). Mapping of QTL underlying tolerance to alkali at germination and early seedling stages in rice. *Acta Agronomica Sinica*, 34(10), 1719-1727.
- Cheng, L., Wang, Y., Meng, L., Hu, X., Cui, Y., Sun, Y., Zhu, L., Ali, J., Xu, J., and Li, Z. (2011). Identification of salt-tolerant QTLs with strong genetic background effect using two sets of reciprocal introgression lines in rice. *Genome*, 55(1), 45-55.
- Cho, Y. S. (2010). Germination characteristics of Korean and Southeast Asian redrice (*Oryza sativa* L.) seeds as affected by temperature. *Asian Journal of Plant Sciences*, 9(2), 104.
- Choudhury, S., Panda, P., Sahoo, L., and Panda, S. K. (2013). Reactive oxygen species signaling in plants under abiotic stress. *Plant Signaling & Behavior*, 8(4), e23681.
- Churchill, G. A., and Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics*, 138(3), 963-971.

- Collard, B., Septiningsih, E., Das, S., Carandang, J., Sanchez, D., Kato, Y., Ye, G., Reddy, J., Singh, U., and Aula, K. (2013). Developing new flood-tolerant varieties at the International Rice Research Institute (IRRI). *SABRAO Journal of Breeding & Genetics*, 45(1), 42-56.
- Collard, B. C. Y., Jahufer, M. Z. Z., Brouwer, J. B., and Pang, E. C. K. (2005). An introduction to markers, quantitative trait loci (QTL) mapping and marker-assisted selection for crop improvement: the basic concepts. *Euphytica*, 142(1-2), 169-196.
- Collard, B. C. Y., and Mackill, D. J. (2008). Marker-assisted selection: An approach for precision plant breeding in the twenty-first century. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1491), 557-572.
- Cramer, G. R., Urano, K., Delrot, S., Pezzotti, M., and Shinozaki, K. (2011). Effects of abiotic stress on plants: a systems biology perspective. *BMC Plant Biology*, 11(1), 163.
- Cruz, R. P. D., and Milach, S. C. K. (2004). Cold tolerance at the germination stage of rice: methods of evaluation and characterization of genotypes. *Scientia Agricola*, 61(1), 1-8.
- Cui, D., Xu, C. Y., Yang, C. G., Zhang, Q. X., Zhang, J. G., Ma, X. D., Qiao, Y. L., Cao, G. L., Zhang, S. Y., and Han, L. Z. (2015). Association mapping of salinity and alkalinity tolerance in improved *Japonica* rice (*Oryza sativa* L. subsp. *japonica* Kato) germplasm. *Genetic Resources and Crop Evolution*, 62(4), 539-550.
- Datta, K., Baisakh, N., Ganguly, M., Krishnan, S., Yamaguchi, S. K., and Datta, S. K. (2012). Overexpression of Arabidopsis and Rice stress genes' inducible transcription factor confers drought and salinity tolerance to rice. *Plant Biotechnology Journal*, 10(5), 579-586.
- Deng, X. X., Zhang, X. Q., Song, X. J., Lai, K. K., Guo, L. B., Xin, Y. Y., Wang, H. Z., and Xue, D. W. (2011). Response of transgenic rice at germination traits under salt and alkali stress. *African Journal of Agricultural Research*, 6(18), 4335-4339.
- Doerge, R. W. (2002). Mapping and analysis of quantitative trait loci in experimental populations. *Nature Reviews Genetics*, 3(1), 43-52.

- Dongre, A. B., Raut, M. P., Bhandarkar, M. R., and Meshram, K. J. (2011). Identification and genetic purity testing of cotton F<sub>1</sub> hybrid using molecular markers. *Indian J Biotechnol*, 10(1), 301-306.
- Dubcovsky, J., Santa, M. G., Epstein, E., Luo, M. C., and Dvořák, J. (1996). Mapping of the K<sup>+</sup>/Na<sup>+</sup> discrimination locus Kna1 in wheat. *Theoretical and Applied Genetics*, 92(3-4), 448-454.
- Dufey, I., Draye, X., Lutts, S., Lorieux, M., Martinez, C., and Bertin, P. (2015). Novel QTLs in an interspecific backcross *Oryza sativa* × *Oryza glaberrima* for resistance to iron toxicity in rice. *Euphytica*, 204(3), 609-625.
- Duque, A. S., Farinha, A. P., da Silva, A. B., de Almeida, A. M., Santos, D., da Silva, J. M., Fevereiro, P., and de Sousa Araújo, S. (2013). *Abiotic stress responses in plants: unraveling the complexity of genes and networks to survive*: INTECH Open Access Publisher.
- Dwivedi, S. L., Ceccarelli, S., Blair, M. W., Upadhyaya, H. D., Are, A. K., and Ortiz, R. (2016). Landrace germplasm for improving yield and abiotic stress adaptation. *Trends in Plant Science*, 21(1), 31-42.
- El Goumi, Y., Fakiri, M., Lamsaouri, O., and Benchekroun, M. (2014). Salt stress effect on seed germination and some physiological traits in three Moroccan barley (*Hordeum vulgare* L.) cultivars. *J. Mater. Environ. Sci*, 5(2), 625-632.
- Evenson, R. E., and Gollin, D. (2003). Assessing the impact of the Green Revolution, 1960 to 2000. *Science*, 300(5620), 758-762.
- Fairhurst, T., Witt, C., Buresh, R., and Dobermann, A. (2007). *Rice: A practical guide to nutrient management*. Los Banos, Philippines: International Rice Research Institute (IRRI) pp191.
- Fan, C., Xing, Y., Mao, H., Lu, T., Han, B., Xu, C., Li, X., and Zhang, Q. (2006). GS3, a major QTL for grain length and weight and minor QTL for grain width and thickness in rice, encodes a putative transmembrane protein. *Theoretical and Applied Genetics*, 112(6), 1164-1171.
- FAO. (2010). Rice Market Monitor. *Trade and Markets Division XIII*(3), 37.
- FAO. (2016). FAO rice market monitor (Retrieved September 2016, from FAO: <http://www.fao.org/economic/RMM/>)
- Farooq, M., Siddique, K. H., Rehman, H., Aziz, T., Lee, D. J., and Wahid, A. (2011). Rice direct seeding: experiences, challenges and opportunities. *Soil and Tillage Research*, 111(2), 87-98.

- Flowers, T. J. (2004). Improving crop salt tolerance. *Journal of Experimental Botany*, 55(396), 307-319.
- Flowers, T. J., and Flowers, S. A. (2005). Why does salinity pose such a difficult problem for plant breeders? *Agricultural Water Management*, 78(1), 15-24.
- Flowers, T. J., Koyama, M. L., Flowers, S. A., Sudhakar, C., Singh, K. P., and Yeo, A. R. (2000). QTL: Their place in engineering tolerance of rice to salinity. *Journal of Experimental Botany*, 51(342), 99-106.
- Flowers, T. J., and Yeo, A. R. (1995). Breeding for salinity resistance in crop plants: where next? *Functional Plant Biology*, 22(6), 875-884.
- Gardi, C., Panagos, P., Van Liedekerke, M., Bosco, C., and De Brogniez, D. (2015). Land take and food security: assessment of land take on the agricultural production in Europe. *Journal of Environmental Planning and Management*, 58(5), 898-912.
- Gardner, G. (1996). *Shrinking fields: cropland loss in a world of eight billion*. Washington, DC: Worldwatch Institute. pp56.
- Ghassemi, F., Jakeman, A. J., and Nix, H. A. (1995). *Salinisation of land and water resources: Human causes, extent, management and case studies*: CAB International, Wallingford, Oxon, UK. pp 526.
- Gong, J., He, P., Qian, Q., Shen, L., Zhu, L., and Chen, S. (1999). Identification of salt-tolerance QTL in rice (*Oryza sativa* L.). *Chinese Science Bulletin*, 44(1), 68-71.
- Grattan, S. R., and Grieve, C. M. (1992). Mineral element acquisition and growth response of plants grown in saline environments. *Agriculture, Ecosystems & Environment*, 38(4), 275-300.
- Gregorio, G., and Senadhira, D. (1993). Genetic analysis of salinity tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 86(2-3), 333-338.
- Gregorio, G. B., Senadhira, D., and Mendoza, R. D. (1997). Screening rice for salinity tolerance. *IRRI DAPO Box 7777, Metro Manila, The Philippines*, 3 - 19.
- Guo, R., Shi, L., Ding, X., Hu, Y., Tian, S., Yan, D., Shao, S., Gao, Y., Liu, R., and Yang, Y. (2010). Effects of saline and alkaline stress on germination, seedling growth, and ion balance in wheat. *Agronomy Journal*, 102(4), 1252-1260.



- Guo, R., Shi, L., and Yang, Y. (2009). Germination, growth, osmotic adjustment and ionic balance of wheat in response to saline and alkaline stresses. *Soil Science and Plant Nutrition*, 55(5), 667-679.
- Gupta, R. K., Naresh, R. K., Hobbs, P. R., Jianguo, Z., and Ladha, J. K. (2003). Sustainability of post-green revolution agriculture: the rice–wheat cropping systems of the Indo-Gangetic Plains and China. *Improving the productivity and sustainability of rice–wheat systems: Issues and impacts*(improving the pro), 1-25.
- Gupta, S. K., and Sharma, S. K. (1990). Response of crops to high exchangeable sodium percentage. *Irrigation Science*, 11(3), 173-179.
- Hai, L., Li, X. G., Li, F. M., Suo, D. R., and Guggenberger, G. (2010). Long-term fertilization and manuring effects on physically-separated soil organic matter pools under a wheat–wheat–maize cropping system in an arid region of China. *Soil Biology and Biochemistry*, 42(2), 253-259.
- Hakim, M., Juraimi, A. S., Begum, M., Hanafi, M., Ismail, M. R., and Selamat, A. (2010). Effect of salt stress on germination and early seedling growth of rice (*Oryza sativa* L.). *African Journal of Biotechnology*, 9(13), 1911-1918.
- Haq, T. U., Gorham, J., Akhtar, J., Akhtar, N., and Steele, K. A. (2009). Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. *Functional Plant Biology*, 37(7), 634-645.
- Haq, T. U., Gorham, J., Akhtar, J., Akhtar, N., and Steele, K. A. (2010). Dynamic quantitative trait loci for salt stress components on chromosome 1 of rice. *Functional Plant Biology*, 37(7), 634-645.
- Hariadi, Y. C., Nurhayati, A. Y., Soeparjono, S., and Arif, I. (2015). Screening Six Varieties of Rice (*Oryza sativa*) for Salinity Tolerance. *Procedia Environmental Sciences*, 28(1), 78-87.
- Hashim, G. M. (2003). *Salt-affected soils of Malaysia*. Paper presented at the Proceedings of the Workshop on Soil Science in Malaysia towards 2020.
- Hashizume, T., Shimamoto, I., and Hirai, M. (2003). Construction of a linkage map and QTL analysis of horticultural traits for watermelon [*Citrullus lanatus* (THUNB.) MATSUM & NAKAI] using RAPD, RFLP and ISSR markers. *Theoretical and Applied Genetics*, 106(5), 779-785.

- Hedayati, P., Monfard, H. H., Isa, N. M., Hwang, D. J., Zain, C. R. C. M., Uddin, M. I., Ismail, I., and Zainal, Z. (2015). Construction and analysis of an *Oryza sativa* (cv. MR219) salinity-related cDNA library. *Acta Physiologiae Plantarum*, 37(5), 1-11.
- Heenan, D., Lewin, L., and McCaffery, D. (1988). Salinity tolerance in rice varieties at different growth stages. *Animal Production Science*, 28(3), 343-349.
- Hizbai, B. T., Gardner, K., Wight, C., Dhanda, R., Molnar, S., Johnson, D., Fregeau-Reid, J., Yan, W., Rossnagel, B., and Holland, J. (2012). Quantitative trait loci affecting oil content, oil composition, and other agronomically important traits in oat. *The Plant Genome*, 5(3), 164-175.
- Hoang, T. M., Moghaddam, L., Williams, B., Khanna, H., Dale, J., and Mundree, S. G. (2015). Development of salinity tolerance in rice by constitutive-overexpression of genes involved in the regulation of programmed cell death. *Frontiers in Plant Science*, 6(1), 175.
- Horie, T., Karahara, I., and Katsuhara, M. (2012). Salinity tolerance mechanisms in glycophytes: An overview with the central focus on rice plants. *Rice*, 5(1), 1.
- Hossain, M. (1997). Rice supply and demand in Asia: a socioeconomic and biophysical analysis. In *Applications of Systems Approaches at the Farm and Regional Levels* Springer (1)263-279
- Hosseini, S. J., Tahmasebi, S. Z., and Pirdashti, H. (2012). Analysis of tolerance indices in some rice (*Oryza sativa* L.) genotypes at salt stress condition. *International Research Journal of Applied and Basic Sciences*, 3(1), 1-10.
- Hu, J., Xiao, C., Cheng, M. X., Gao, G. J., Zhang, Q. L., and He, Y. Q. (2015). A new finely mapped *Oryza australiensis*-derived QTL in rice confers resistance to brown planthopper. *Gene*, 561(1), 132-137.
- Huang, N., Parco, A., Mew, T., Magpantay, G., McCouch, S., Guiderdoni, E., Xu, J., Subudhi, P., Angeles, E. R., and Khush, G. S. (1997). RFLP mapping of isozymes, RAPD and QTLs for grain shape, brown planthopper resistance in a doubled haploid rice population. *Molecular Breeding*, 3(2), 105-113.
- Ikeda, N., Bautista, N. S., Yamada, T., Kamijima, O., and Ishii, T. (2001). Ultra-simple DNA extraction method for marker-assisted selection using microsatellite markers in rice. *Plant Molecular Biology Reporter*, 19(1), 27-32.

- Imran, M., Mahmood, A., Römheld, V., and Neumann, G. (2013). Nutrient seed priming improves seedling development of maize exposed to low root zone temperatures during early growth. *European Journal of Agronomy*, 49, 141-148.
- IRRI. (1986). *Rice Genetics: Proceedings of the International Rice Genetics Symposium, 27-31 May 1985*: International Rice Research Institute. 399-409.
- IRRI. (2011). Stress and disease tolerance. *International Rice Research Institute* (Retrieved on December 23, 2016 from, [http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding\\_for\\_salt\\_tolerance.htm](http://www.knowledgebank.irri.org/ricebreedingcourse/Breeding_for_salt_tolerance.htm)).
- Islam, M. R., Salam, M. A., Hassan, L., Collard, B. C. Y., Singh, R. K., and Gregorio, G. B. (2011). QTL mapping for salinity tolerance at seedling stage in rice. *Emirates Journal of Food and Agriculture*, 23(2), 137.
- Islam, M. Z., Baset Mia, M. A., Islam, M. R., and Akter, A. (2007). Effect of different saline levels on growth and yield attributes of mutant rice. *J Soil Nat*, 1(2), 18-22.
- Ismail, A. M., Singh, U. S., Singh, S., Dar, M. H., and Mackill, D. J. (2013). The contribution of submergence-tolerant (Sub1) rice varieties to food security in flood-prone rainfed lowland areas in Asia. *Field Crops Research*, 152(1), 83-93.
- Jamil, M., Bashir, S., Anwar, S., Bibi, S., Bangash, A., Ullah, F., and Rha, E. S. (2012). Effect of salinity on physiological and biochemical characteristics of different varieties of rice. *Pak J Bot*, 44, 7-13.
- Javed, M. A., Huyop, F. Z., Ishii, T., ABD A, S. T., Haider, M., and Saleem, M. (2013). Construction of microsatellite linkage map and detection of segregation distortion in *Indica* rice (*Oryza sativa* L.). *Pakistan J Bot*, 45, 2085-2092.
- Javed, M. A., Huyop, F. Z., Wagiran, A., and Salleh, F. M. (2011). Identification of QTLs for morph-physiological traits related to salinity tolerance at seedling stage in *Indica* rice. *Procedia Environmental Sciences*, 8(1), 389-395.
- Jena, K. K. (2010). The species of the genus *Oryza* and transfer of useful genes from wild species into cultivated rice, *O. sativa*. *Breeding Science*, 60(5), 518-523.
- Jiang, L., and Li, Z. (2016). Urbanization and the Change of Fertilizer Use Intensity for Agricultural Production in Henan Province. *Sustainability*, 8(2), 186.

- Jones, M. P. (1985). *Genetic analysis of salt tolerance in mangrove swamp rice*. Paper presented at the Rice Genetics Proceeding of International Rice Genetics Symposium, IRRI, 41-122.
- Joseph, B., Jini, D., and Sujatha, S. (2010). Biological and physiological perspectives of specificity in abiotic salt stress response from various rice plants. *Asian J. Agric. Sci*, 2(3), 99-105.
- Kandil, A. A., Sharief, A. E., and Nassar, E. S. E. (2012). Response of some rice (*Oryza sativa* L.) cultivars to germination under salinity stress. *International Journal of Agriculture Sciences*, 4(6), 272-277.
- Kang, S., Post, W. M., Nichols, J. A., Wang, D., West, T. O., Bandaru, V., and Izaurralde, R. C. (2013). Marginal lands: concept, assessment and management. *Journal of Agricultural Science*, 5(5), 129.
- Kanjoo, V., Jearakongman, S., Punyawaew, K., Siangliw, J. L., Siangliw, M., Vanavichit, A., and Toojinda, T. (2011). Co-location of quantitative trait loci for drought and salinity tolerance in rice. *Thai J. Genet*, 4(2), 126-138.
- Kao, C.-H., Zeng, Z.-B., and Teasdale, R. D. (1999). Multiple interval mapping for quantitative trait loci. *Genetics*, 152(3), 1203-1216.
- Karim, M. A., Utsunomiya, N., and Shigenaga, S. (1992). Effect of Sodium Chloride on Germination and Growth of Hexaploid Triticale at Early Seedling Stage. *Japanese Journal of Crop Science*, 61(2), 279-284.
- Khan, M., Hamid, A., and Karim, M. (1997). Effect of sodium chloride on germination and seedling characters of different types of rice (*Oryza sativa* L.). *Journal of Agronomy and Crop Science*, 179(3), 163-169.
- Khan, M. A., and Abdullah, Z. (2003). Salinity–sodicity induced changes in reproductive physiology of rice (*Oryza sativa*) under dense soil conditions. *Environmental and Experimental Botany*, 49(2), 145-157.
- Khan, M. A., and Rizvi, Y. (1994). Effect of salinity, temperature, and growth regulators on the germination and early seedling growth of *Atriplex griffithii* var. stocksii. *Canadian Journal of Botany*, 72(4), 475-479.
- Khan, S., Javed, M. A., Jahan, N., and Manan, F. A. (2016). A Short Review On The Development Of Salt Tolerant Cultivars In Rice. *International Journal of Public Health Science (IJPHS)*, 5(2).
- Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. In *Oryza: From molecule to plant* Springer 25-34

- Kim, H., Hwang, H., Hong, J.-W., Lee, Y.-N., Ahn, I. P., Yoon, I. S., Yoo, S.-D., Lee, S., Lee, S. C., and Kim, B.-G. (2012). A rice orthologue of the ABA receptor, OsPYL/RCAR5, is a positive regulator of the ABA signal transduction pathway in seed germination and early seedling growth. *Journal of Experimental Botany*, 63(2), 1013-1024.
- Kimi, S., and Daham, M. D. (1993). *Managing salinity problems in rice fields*. Paper presented at the Proceedings of the International Conference for Agricultural Machinery and Process Engineering, Seoul, Korea, 556-564.
- Kosambi, D. D. (1943). The estimation of map distances from recombination values. *Annals of Eugenics*, 12(1), 172-175.
- Kovach, M. J., Sweeney, M. T., and McCouch, S. R. (2007). New insights into the history of rice domestication. *TRENDS in Genetics*, 23(11), 578-587.
- Koyama, M. L., Levesley, A., Koebner, R. M., Flowers, T. J., and Yeo, A. R. (2001). Quantitative trait loci for component physiological traits determining salt tolerance in rice. *Plant Physiology*, 125(1), 406-422.
- Kukul, S., and Aggarwal, G. (2002). Percolation losses of water in relation to puddling intensity and depth in a sandy loam rice (*Oryza sativa*) field. *Agricultural Water Management*, 57(1), 49-59.
- Kumar, A., Dixit, S., Ram, T., Yadaw, R., Mishra, K., and Mandal, N. (2014). Breeding high-yielding drought-tolerant rice: genetic variations and conventional and molecular approaches. *Journal of Experimental Botany*, 65(21), 6265-6278.
- Kumar, R., Mustafiz, A., Sahoo, K. K., Sharma, V., Samanta, S., Sopory, S. K., Pareek, A., and Singla-Pareek, S. L. (2012). Functional screening of cDNA library from a salt tolerant rice genotype *Pokkali* identifies mannose-1-phosphate guanyl transferase gene (OsMPG1) as a key member of salinity stress response. *Plant molecular biology*, 79(6), 555-568.
- Kumar, V., Shriram, V., Nikam, T., Jawali, N., and Shitole, M. G. (2009). Antioxidant enzyme activities and protein profiling under salt stress in *Indica* rice genotypes differing in salt tolerance. *Archives of Agronomy and Soil Science*, 55(4), 379-394.

- Kumar, V., Singh, A., Mithra, S. A., Krishnamurthy, S., Parida, S. K., Jain, S., Tiwari, K. K., Kumar, P., Rao, A. R., and Sharma, S. (2015). Genome-wide association mapping of salinity tolerance in rice (*Oryza sativa*). *DNA Research*, 22(2), 133-145.
- Lander, E. S., and Botstein, D. (1989). Mapping mendelian factors underlying quantitative traits using RFLP linkage maps. *Genetics*, 121(1), 185-199.
- Lang, N. T., Yanagihara, S., and Buu, B. C. (2001). QTL analysis of salt tolerance in rice (*Oryza sativa* L.). *SABRAO Journal of Breeding and Genetics*, 33(1), 11-20.
- Le, L. H., Tran, K. D., Van, N. L., Dong, C. T. K., Le, D. D., Ta, L. H., Ismail, A. M., and Le, H. H. (2016). Application of Marker Assisted Backcrossing to Pyramid Salinity Tolerance (Saltol) into Rice Cultivar-Bac Thom 7. *VNU Journal of Science: Natural Sciences and Technology*, 28(2), 87-99.
- Lee, K. S., Choi, W. Y., Ko, J. C., Kim, T. S., and Gregorio, G. B. (2003). Salinity tolerance of japonica and *Indica* rice (*Oryza sativa* L.) at the seedling stage. *Planta*, 216(6), 1043-1046.
- Lee, M. H., Cho, E. J., Wi, S. G., Bae, H., Kim, J. E., Cho, J.-Y., Lee, S., Kim, J.-H., and Chung, B. Y. (2013). Divergences in morphological changes and antioxidant responses in salt-tolerant and salt-sensitive rice seedlings after salt stress. *Plant Physiology and Biochemistry*, 70, 325-335.
- Lee, S. Y., Ahn, J. H., Cha, Y. S., Yun, D. W., Lee, M. C., Ko, J. C., Lee, K. S., and Eun, M. Y. (2006). Mapping of quantitative trait loci for salt tolerance at the seedling stage in rice. *Molecules & Cells*, 21(2), 192-196.
- Li, C. B., Zhang, D. M., Ge, S., Lu, B. R., and Hong, D. Y. (2001). Identification of genome constitution of *Oryza malampuzhaensis*, *O. minuta*, and *O. punctata* by multicolor genomic *in situ* hybridization. *Theoretical and Applied Genetics*, 103(2-3), 204-211.
- Li, R., Shi, F., Fukuda, K., and Yang, Y. (2010). Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). *Soil Science & Plant Nutrition*, 56(5), 725-733.
- Li, X., Wang, X., He, K., Ma, Y., Su, N., He, H., Stolc, V., Tongprasit, W., Jin, W., and Jiang, J. (2008). High-resolution mapping of epigenetic modifications of the rice genome uncovers interplay between DNA methylation, histone methylation, and gene expression. *The Plant Cell*, 20(2), 259-276.

- Li, X., Yan, W., Agrama, H., Jackson, A., Jia, M., Jia, L., Moldenhauer, K., Correa, F., and Wu, D. (2016). Genetic analysis of genetic basis of a physiological disorder “straighthead” in rice (*Oryza sativa* L.). *Genes & Genomics*, 38(5), 453-457.
- Li, Z. K. (2001). QTL mapping in rice: a few critical considerations. *In Proc. Fourth Int. Rice Genetics Symp.* (eds G. S. Brar & B. Hardy), Los Banos, The Philippines: International Rice Research Institute. 153-172.
- Liang, J. L., Qu, Y. P., Yang, C. G., Ma, X. D., Cao, G. L., Zhao, Z. W., Zhang, S. Y., Zhang, T., and Han, L. Z. (2015). Identification of QTLs associated with salt or alkaline tolerance at the seedling stage in rice under salt or alkaline stress. *Euphytica*, 201(3), 441-452.
- Lin, H. X., Zhu, M. Z., Yano, M., Gao, J. P., Liang, Z. W., Su, W. A., Hu, X. H., Ren, Z. H., and Chao, D. Y. (2004). QTLs for Na<sup>+</sup> and K<sup>+</sup> uptake of the shoots and roots controlling rice salt tolerance. *Theoretical and Applied Genetics*, 108(2), 253-260.
- Lin, S. Y., Sasaki, T., and Yano, M. (1998). Mapping quantitative trait loci controlling seed dormancy and heading date in rice, *Oryza sativa* L., using backcross inbred lines. *Theoretical and Applied Genetics*, 96(8), 997-1003.
- Lin, Z. X., He, D., Zhang, X.-l., Nie, Y., Guo, X., Feng, C., and Stewart, J. M. (2005). Linkage map construction and mapping QTL for cotton fibre quality using SRAP, SSR and RAPD. *Plant breeding*, 124(2), 180-187.
- Lincoln, S., Daly, M., and Lander, E. (1992). Constructing genetics maps with MAPMAKER/EXP 3.0. Whitehead Institute, Cambridge, MA.
- Lincoln, S. E., Daly, M. J., and Lander, E. S. (1993). Constructing genetic linkage maps with MAPMAKER/EXP Version 3.0: a tutorial and reference manual. *A Whitehead Institute for Biomedical Research Technical Report*, 78-79.
- Lindsay, M. P., Lagudah, E. S., Hare, R. A., and Munns, R. (2004). A locus for sodium exclusion (Nax1), a trait for salt tolerance, mapped in durum wheat. *Functional Plant Biology*, 31(11), 1105-1114.
- Liu, J., Guo, W., and Shi, D. (2010). Seed germination, seedling survival, and physiological response of sunflowers under saline and alkaline conditions. *Photosynthetica*, 48(2), 278-286.

- Liu, L., Liu, G., Gong, Y., Dai, W., Wang, Y., Yu, F., and Ren, Y. (2007). Evaluation of genetic purity of F<sub>1</sub> hybrid seeds in cabbage with RAPD, ISSR, SRAP, and SSR markers. *HortScience*, 42(3), 724-727.
- Luo, X., Ji, S. D., Yuan, P. R., Lee, H. S., Kim, D. M., Balkunde, S., Kang, J. W., and Ahn, S. N. (2013). QTL mapping reveals a tight linkage between QTLs for grain weight and panicle spikelet number in rice. *Rice*, 6(1), 1.
- Lutts, S., Kinet, J. M., and Bouharmont, J. (1995). Changes in plant response to NaCl during development of rice (*Oryza sativa* L.) varieties differing in salinity resistance. *Journal of Experimental Botany*, 46(12), 1843-1852.
- Lv, B. S., Li, X. W., Ma, H. Y., Sun, Y., Wei, L. X., Jiang, C. J., and Liang, Z. W. (2013). Differences in growth and physiology of rice in response to different saline-alkaline stress factors. *Agronomy Journal*, 105(4), 1119-1128.
- Lv, H. Y., Liang, Z. W., Lv, B. S., and Yin, H. B. (2015). Responses of rice (*Oryza sativa* L.) mutant ML04 and its wild type seedling growth to saline-alkaline stress. *Research on Crops*, 16(1), 1-14.
- Ma, H., Yang, H., Lü, X., Pan, Y., Wu, H., Liang, Z., and Ooi, M. K. (2015). Does high pH give a reliable assessment of the effect of alkaline soil on seed germination? A case study with *Leymus chinensis* (Poaceae). *Plant and Soil*, 394(1-2), 35-43.
- Maccaferri, M., Sanguineti, M. C., Corneti, S., Ortega, J. L. A., Salem, M. B., Bort, J., DeAmbrogio, E., del Moral, L. F. G., Demontis, A., and El-Ahmed, A. (2008). Quantitative trait loci for grain yield and adaptation of durum wheat (*Triticum durum* Desf.) across a wide range of water availability. *Genetics*, 178(1), 489-511.
- Mackill, D. J., Ismail, A. M., Singh, U. S., Labios, R. V., and Paris, T. R. (2012). Development and Rapid Adoption of Submergence-Tolerant (Sub1) Rice Varieties. *Advances in Agronomy*, 115(1), 303-356.
- Madesis, P., Ganopoulos, I., and Tsaftaris, A. (2013). Microsatellites: evolution and contribution. *Method Molecular Biology* 1006:1-16.
- Mahmood, T., Turner, M., Stoddard, F. L., and Javed, M. A. (2004). Genetic analysis of quantitative traits in rice (*Oryza sativa* L.) exposed to salinity. *Crop and Pasture Science*, 55(11), 1173-1181.



- Manly, K. F., Cudmore Jr, R. H., and Meer, J. M. (2001). Map Manager QTX, cross-platform software for genetic mapping. *Mammalian Genome*, 12(12), 930-932.
- Manly, K. F., and Olson, J. M. (1999). Overview of QTL mapping software and introduction to Map Manager QT. *Mammalian Genome*, 10(4), 327-334.
- Manrique-Carpintero, N. C., Coombs, J. J., Veilleux, R. E., Buell, C. R., and Douches, D. S. (2016). Comparative Analysis of Regions with Distorted Segregation in Three Diploid Populations of Potato. *G3: Genes Genomes Genetics*, 6(8), 2617-2628.
- Mansuri, S. M., Jelodar, N. B., and Bagheri, N. (2012). Evaluation of rice genotypes to salt stress in different growth stages via phenotypic and random amplified polymorphic DNA (RAPD) marker assisted selection. *African Journal of Biotechnology*, 11(39), 9362-9372.
- Marco, F., Bitrián, M., Carrasco, P., Rajam, M. V., Alcázar, R., and Tiburcio, A. F. (2015). Genetic engineering strategies for abiotic stress tolerance in plants. In *Plant Biology and Biotechnology* Springer, 579-609.
- Mardani, Z., Rabiei, B., Sabouri, H., and Sabouri, A. (2014). Identification of molecular markers linked to salt-tolerant genes at germination stage of rice. *Plant Breeding*, 133(2), 196-202.
- Marie-Vivien, D. (2008). From plant variety definition to geographical indication protection: A search for the link between Basmati rice and India/Pakistan. *The Journal of World Intellectual Property*, 11(4), 321-344.
- Masood, M. S., Seiji, Y., Shinwari, Z. K., and Anwar, R. (2004). Mapping quantitative trait loci (QTLs) for salt tolerance in rice (*Oryza sativa*) using RFLPs. *Pak. J. Bot*, 36(4), 825-834.
- Matsuo, T., Futsuhara, Y., Kikuchi, F., and Yamaguchi, H. (1997). *Science of the rice plant. 3. Genetics. Supplementary volume: indices (Vol. 3)*: Food and Agriculture Policy Research Center. 302-303.
- McCouch, S., Kochert, G., Yu, Z., Wang, Z., Khush, G., Coffman, W., and Tanksley, S. (1988). Molecular mapping of rice chromosomes. *Theoretical and Applied Genetics*, 76(6), 815-829.
- McCough, S. R., and Doerge, R. W. (1995). QTL mapping in rice. *Trends in Genetics*, 11(12), 482-487.

- McCouch, S. R., Teytelman, L., Xu, Y., Lobos, K. B., Clare, K., Walton, M., Fu, B., Maghirang, R., Li, Z., and Xing, Y. (2002). Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.). *DNA research*, 9(6), 199-207.
- Miah, G., Rafii, M. Y., Ismail, M. R., Puteh, A. B., Rahim, H. A., Islam, K. N., and Latif, M. A. (2013). A review of microsatellite markers and their applications in rice breeding programs to improve blast disease resistance. *International journal of molecular sciences*, 14(11), 22499-22528.
- Mirosavljević, M., Čanak, P., Ćirić, M., Nastasić, A., Dukić, D., and Rajković, M. (2013). Maize germination parameters and early seedlings growth under different levels of salt stress. *Ratarstvo i Povrtarstvo*, 50(1), 49-53.
- Mittler, R. (2006). Abiotic stress, the field environment and stress combination. *Trends in Plant Science*, 11(1), 15-19.
- Mohammad, R. M., Campbell, W., and Rumbaugh, M. (1989). Variation in salt tolerance of alfalfa. *Arid Land Research and Management*, 3(1), 11-20.
- Mohammadi, R., Mendioro, M. S., Diaz, G. Q., Gregorio, G. B., and Singh, R. K. (2014). Genetic analysis of salt tolerance at seedling and reproductive stages in rice (*Oryza sativa*). *Plant Breeding*, 133(5), 548-559.
- Mondal, T., Bal, A., and Pal, S. (1988). Effect of salinity on germination and seedling growth of different rice (*Oryza sativa* L.) cultivars. *J. Indian Soc. Coast. Agric. Res*, 6(1), 91-97.
- Moorby, J., and Besford, R. T. (1983). Mineral nutrition and growth. *Encyclopedia of Plant Physiology New Series*, 15(1), 481-515.
- Moretzsohn, M. C., Gouvea, E. G., Inglis, P. W., Leal-Bertioli, S. C., Valls, J. F., and Bertioli, D. J. (2013). A study of the relationships of cultivated peanut (*Arachis hypogaea*) and its most closely related wild species using intron sequences and microsatellite markers. *Annals of botany*, 111(1), 113-126.
- Morin, J., Benyamini, Y., and Michaeli, A. (1981). The effect of raindrop impact on the dynamics of soil surface crusting and water movement in the profile. *Journal of Hydrology*, 52(3), 321-335.
- Mueller, U. G., and Wolfenbarger, L. L. (1999). AFLP genotyping and fingerprinting. *Trends in Ecology & Evolution*, 14(10), 389-394.
- Munns, R., and Tester, M. (2008). Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.*, 59(1), 651-681.

- Nakashima, K., Jan, A., Todaka, D., Maruyama, K., Goto, S., Shinozaki, K., and Yamaguchi-Shinozaki, K. (2014). Comparative functional analysis of six drought-responsive promoters in transgenic rice. *Planta*, 239(1), 47-60.
- Nandakumar, N., Singh, A., Sharma, R., Mohapatra, T., Prabhu, K., and Zaman, F. (2004). Molecular fingerprinting of hybrids and assessment of genetic purity of hybrid seeds in rice using microsatellite markers. *Euphytica*, 136(3), 257-264.
- Nandi, S., Subudhi, P. K., Senadhira, D., Manigbas, N. L., Sen-Mandi, S., and Huang, N. (1997). Mapping QTLs for submergence tolerance in rice by AFLP analysis and selective genotyping. *Molecular and General Genetics MGG*, 255(1), 1-8.
- Nath, R., Luan, Y., Yang, W., Yang, C., Chen, W., Li, Q., and Cui, X. (2015). Changes in arable land demand for food in India and China: A potential threat to food security. *Sustainability*, 7(5), 5371-5397.
- Negrao, S., Courtois, B., Ahmadi, N., Abreu, I., Saibo, N., and Oliveira, M. M. (2011). Recent updates on salinity stress in rice: from physiological to molecular responses. *Critical Reviews in Plant Sciences*, 30(4), 329-377.
- Nguyen, N. (2002). Global climate changes and rice food security. *Rome, Italy: FAO*.
- Niones, J. M., Inukai, Y., Suralta, R. R., and Yamauchi, A. (2015). QTL associated with lateral root plasticity in response to soil moisture fluctuation stress in rice. *Plant and Soil*, 391(1-2), 63-75.
- Noorzuraini, A., Borromeo, T., Nestor, N., Diaz, G., and Arvind, K. (2013). Diversity assessment of Malaysian rice germplasm accessions for drought tolerant grain yield QTLs. *J Trop Agric*, 41, 27-40.
- Oka, H. I. (2012). *Origin of cultivated rice* (Vol. 14). Amsterdam: Elsevier. 25.
- Ologundudu, A. F., Adelusi, A. A., and Akinwale, R. O. (2014). Effect of Salt Stress on Germination and Growth Parameters of Rice. *Notulae Scientia Biologicae*, 6(2), 237.
- Pandey, G., Misra, G., Kumari, K., Gupta, S., Parida, S. K., Chattopadhyay, D., and Prasad, M. (2013). Genome-wide development and use of microsatellite markers for large-scale genotyping applications in foxtail millet [*Setaria italica* (L.)]. *DNA research*, 20(2), 197-207.

- Pandit, A., Rai, V., Bal, S., Sinha, S., Kumar, V., Chauhan, M., Gautam, R. K., Singh, R., Sharma, P. C., and Singh, A. K. (2010). Combining QTL mapping and transcriptome profiling of bulked RILs for identification of functional polymorphism for salt tolerance genes in rice (*Oryza sativa* L.). *Molecular Genetics and Genomics*, 284(2), 121-136.
- Park, Y. J., Lee, J. K., and Kim, N. S. (2009). Simple sequence repeat polymorphisms (SSRPs) for evaluation of molecular diversity and germplasm classification of minor crops. *Molecules*, 14(11), 4546-4569.
- Paz, R. C., Rocco, R. A., Reinoso, H., Menéndez, A. B., Pieckenstain, F. L., and Ruiz, O. A. (2012). Comparative study of alkaline, saline, and mixed saline-alkaline stresses with regard to their effects on growth, nutrient accumulation, and root morphology of *Lotus tenuis*. *Journal of Plant Growth Regulation*, 31(3), 448-459.
- Pessaraki, M., Singh, R. K., and Flowers, T. J. (2010). Physiology and molecular biology of the effects of salinity on rice. In *Handbook of Plant and Crop Stress, Third Edition* CRC Press 899-939.
- Pradheeban, L., Nissanka, N., and Suriyagoda, L. D. B. (2014). Clustering of Rice (*Oryza sativa* L.) Varieties Cultivated in Jaffna District of Sri Lanka based on Salt Tolerance during Germination and Seedling Stages. *Tropical Agricultural Research*, 25(3), 358-375.
- Prasad, S. R., Bagali, P. G., Hittalmani, S., and Shashidhar, H. E. (2000). Molecular mapping of quantitative trait loci associated with seedling tolerance to salt stress in rice (*Oryza sativa* L.). *Current Science*, 78(2), 162-164.
- Prince, S. J., Beena, R., Gomez, S. M., Senthivel, S., and Babu, R. C. (2015). Mapping consistent rice (*Oryza sativa* L.) yield QTLs under drought stress in target rainfed environments. *Rice*, 8(1), 1.
- Project, I. R. G. S. (2005). The map-based sequence of the rice genome. *Nature*, 436(7052), 793-800.
- Qadir, M., Oster, J., Schubert, S., Noble, A., and Sahrawat, K. (2007). Phytoremediation of sodic and saline-sodic soils. *Advances in Agronomy*, 96, 197-247.
- Rad, H., Aref, F., and Rezaei, M. (2012). Evaluation of salinity stress affects rice growth and yield components in Northern Iran. *American Journal of Scientific Research*, 54, 40-51.

- Rahman, M., Miyake, H., and Taheoka, Y. (2001). Effect of sodium chloride salinity on seed germination and early seedling growth of rice (*Oryza sativa* L.). *Pak. J. Biol. Sci.*, 4(3), 351-355.
- Rahman, M. A., Thomson, M. J., Shah-E-Alam, M., de Ocampo, M., Egdane, J., and Ismail, A. M. (2016). Exploring novel genetic sources of salinity tolerance in rice through molecular and physiological characterization. *Annals of Botany*, 1(1), 1-15.
- Rajamoorthy, Y., and Munusamy, S. (2015). Rice industry in Malaysia: challenges, policies and implications. *Procedia Economics and Finance*, 31, 861-867.
- Rajendran, K., Tester, M., and Roy, S. J. (2009). Quantifying the three main components of salinity tolerance in cereals. *Plant, Cell & Environment*, 32(3), 237-249.
- Randhawa, G. J., Verma, D. D., Bhalla, S., Hota, M., Chalam, V. C., and Tyagi, V. (2006). Document on biology of rice (*Oryza sativa* L.) in India. *National Bureau of Plant Genetic Resources*, New Delhi, 1(1), 1-88.
- Rao, H., Basha, O., Singh, N., Sato, K., and Dhaliwal, H. (2007). Frequency distributions and composite interval mapping for QTL analysis in 'Steptoe'x 'Morex'barley mapping population. *Barley Genetics Newsletter*, 37, 5-20.
- Rao, P. S., Mishra, B., Gupta, S. R., and Rathore, A. (2008). Reproductive stage tolerance to salinity and alkalinity stresses in rice genotypes. *Plant Breeding*, 127(3), 256-261.
- Ren, Z. H., Gao, J. P., Li, L. G., Cai, X. L., Huang, W., Chao, D. Y., Zhu, M. Z., Wang, Z. Y., Luan, S., and Lin, H. X. (2005). A rice quantitative trait locus for salt tolerance encodes a sodium transporter. *Nature Genetics*, 37(10), 1141-1146.
- Rieseberg, L. H., Archer, M. A., and Wayne, R. K. (1999). Transgressive segregation, adaptation and speciation. *Heredity*, 83(4), 363-372.
- Rosario, M., Gazaffi, R., Moura, A. S. A. M. T., Ledur, M., Coutinho, L., and Garcia, A. (2014). Composite interval mapping and mixed models reveal QTL associated with performance and carcass traits on chicken chromosomes 1, 3, and 4. *Journal of Applied Genetics*, 55(1), 97-103.
- Sabouri, H., and Sabouri, A. (2008). New evidence of QTLs attributed to salinity tolerance in rice. *African Journal of Biotechnology*, 7(24), 4376-4383.

- Sairam, R. K., and Tyagi, A. (2004). Physiology and molecular biology of salinity stress tolerance in plants. *Current Science Bangalore*, 86(3), 407-421.
- Scherr, S. J. (1999). *Soil Degradation: A Threat to Developing-Country Food Security by 2020?* (Vol. 27): Intl Food Policy Res Inst. pp63.
- Sehgal, D., Singh, R., and Rajpal, V. R. (2016). Quantitative Trait Loci Mapping in Plants: Concepts and Approaches. In *Molecular Breeding for Sustainable Crop Improvement* Springer 31-59.
- Semagn, K., Bjonstad, Å., and Ndjiondjop, M. N. (2006). Progress and prospects of marker assisted backcrossing as a tool in crop breeding programs. *African Journal of Biotechnology*, 5(25), 2588-2603.
- Septiningsih, E. M., Ignacio, J. C. I., Sendon, P. M., Sanchez, D. L., Ismail, A. M., and Mackill, D. J. (2013). QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. *Theoretical and Applied Genetics*, 126(5), 1357-1366.
- Shamsudin, N. A. A., Swamy, B. M., Ratnam, W., Cruz, M. T. S., Raman, A., and Kumar, A. (2016). Marker assisted pyramiding of drought yield QTLs into a popular Malaysian rice cultivar, MR219. *BMC Genetics*, 17(1), 30.
- Shannon, M. C., Grieve, C. M., and Francois, L. E. (1994). *Whole-plant response to salinity*: M Dekker Inc. New York. 199-244
- Sharifzadeh, F., Zolleh, H. H., Mohamadi, H., and Janmohamadi, M. (2006). Study of Osmotic Priming Effects on Wheat (*Triticum aestivum*) Germination in Different Temperatures and Local Seed Masses. *J. of Agronomy*, 5(4), 647-650.
- Sharma, S. K. (1986). Mechanism of tolerance in rice varieties differing in sodicity tolerance. *Plant and Soil*, 93(1), 141-145.
- Shen, X., Guo, W., Zhu, X., Yuan, Y., John, Z. Y., Kohel, R. J., and Zhang, T. (2005). Molecular mapping of QTLs for fiber qualities in three diverse lines in Upland cotton using SSR markers. *Molecular Breeding*, 15(2), 169-181.
- Shi, D., and Wang, D. (2005). Effects of various salt-alkaline mixed stresses on *Aneurolepidium chinense* (Trin.) Kitag. *Plant and Soil*, 271(1-2), 15-26.
- Shi, D. C., and Yin, L. J. (1993). Difference Between Salt (NaCl) and Alkaline (Na<sub>2</sub>CO<sub>3</sub>) Stresses on *Puccinellia tenuiflora* (Griseb.) Scribn. et Merr. *Plants. Acta Botanica Sinica-Chinese Edition*, 35(1), 144-144.

- Silva, L. D. C. E., Wang, S., and Zeng, Z.-B. (2012). Composite interval mapping and multiple interval mapping: procedures and guidelines for using Windows QTL Cartographer. *Quantitative Trait Loci (QTL) Methods and Protocols*, 75-119.
- Simbolo, M., Gottardi, M., Corbo, V., Fassin, M., Mafficini, A., Malpeli, G., Lawlor, R. T., and Scarpa, A. (2013). DNA qualification workflow for next generation sequencing of histopathological samples. *PLoS One*, 8(6), e62692.
- Singh, A., Sharma, P., Gaikwad, K., Sharma, T., Mohapatra, T., and Singh, N. (2009). Mapping of QTLs controlling Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> ion concentrations in salt tolerant *Indica* rice variety CSR27. *J Plant Biochem Biotechnol*, 18(2), 139-150.
- Singh, K., Mishra, A. K., Singh, B., Singh, R. P., and Patra, D. D. (2014). Tillage effects on crop yield and physicochemical properties of sodic soils. *Land Degradation & Development*, 1(1), 1-8.
- Singh, R., and Flowers, T. (2010). 36 The Physiology and Molecular Biology of the Effects of Salinity on Rice. In M. Pessaraki (Ed.), *Handbook of Plant and Crop Stress* Taylor and Francis Florida: 901-942.
- Singh, R. K., Gregorio, G. B., and Jain, R. K. (2007). QTL mapping for salinity tolerance in rice. *Physiology and Molecular Biology of Plants*, 13(1), 87-99.
- Singh, R. K., Redoña, E., and Refuerzo, L. (2009). Varietal improvement for abiotic stress tolerance in crop plants: special reference to salinity in rice. In *Abiotic Stress Adaptation in Plants* Springer 387-415.
- Singh, Y. (2013). Crop and water productivity as influenced by rice cultivation methods under organic and inorganic sources of nutrient supply. *Paddy and Water Environment*, 11(1-4), 531-542.
- Singh, Y., Mishra, V., Singh, S., Sharma, D., Singh, D., Singh, U., Singh, R., Haefele, S., and Ismail, A. (2016). Productivity of sodic soils can be enhanced through the use of salt tolerant rice varieties and proper agronomic practices. *Field Crops Research*, 190(1), 82-90.
- Song, B., Nadarajah, K., and Ratnam, W. (2007). A Non-redundant Strategy for Identification of a Minimum Tiling Path BAC Contig Spanning Approximately 390 kb of the QTL yld1. 1 in *Oryza rufipogon*. *Korean Journal of Genetics*, 29(4), 447.

- Song, B., Nadarajah, K., Romanov, M. N., and Ratnam, W. (2005). Cross-species bacterial artificial chromosome (BAC) library screening via overgo-based hybridization and BAC-contig mapping of a yield enhancement quantitative trait locus (QTL) yld1. 1 in the Malaysian wild rice *Oryza rufipogon*. *Cellular and Molecular Biology Letters*, 10(3), 425.
- Song, B., Wickneswari, R., and Kalaivani, N. (2003). *Development of bac-based physical map for the yield related QTL in Oryza rufipogon*. Paper presented at the 14th Malaysian Society of Plant Physiology Conference, Pahang (Malaysia), 248-253.
- Suzuki, N., Koussevitzky, S., Mittler, R., and Miller, G. (2012). ROS and redox signalling in the response of plants to abiotic stress. *Plant, Cell & Environment*, 35(2), 259-270.
- Syed, M. A., Iftekharruddaula, K., Mian, M. K., Rasul, M. G., Rahmam, G. M., Panaullah, G. M., Lauren, J. G., Duxbury, J. M., and Biswas, P. S. (2016). Main effect QTLs associated with arsenic phyto-toxicity tolerance at seedling stage in rice (*Oryza sativa* L.). *Euphytica*, 209(3), 805-814.
- Takagi, H., Abe, A., Yoshida, K., Kosugi, S., Natsume, S., Mitsuoka, C., Uemura, A., Utsushi, H., Tamiru, M., and Takuno, S. (2013). QTL-seq: rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. *The Plant Journal*, 74(1), 174-183.
- Tanksley, S. D. (1993). Mapping polygenes. *Annual Review of Genetics*, 27(1), 205-233.
- Tanksley, S. D., Young, N. D., Paterson, A. H., and Bonierbale, M. W. (1989). RFLP mapping in plant breeding: new tools for an old science. *Nature Biotechnology*, 7(3), 257-264.
- Teh, C.Y., Shaharuddin, N. A., Ho, C. L., and Mahmood, M. (2016). Exogenous proline significantly affects the plant growth and nitrogen assimilation enzymes activities in rice (*Oryza sativa*) under salt stress. *Acta Physiologiae Plantarum*, 38(6), 1-10.
- Terauchi, R., Abe, A., Takagi, H., Tamiru, M., Fekih, R., Natsume, S., Yaegashi, H., Kosugi, S., Kanzaki, H., and Matsumura, H. (2015). Whole Genome Sequencing to Identify Genes and QTL in Rice. In *Advances in the Understanding of Biological Sciences Using Next Generation Sequencing (NGS) Approaches* Springer 33-42.



- Tester, M., and Bacic, A. (2005). Abiotic stress tolerance in grasses. From model plants to crop plants. *Plant Physiology*, 137(3), 791-793.
- Thomas, R. P., and Morini, S. (2005). Management of irrigation-induced salt-affected soils. *CISAU, IPTRID, AGLL, FAO*. 155-171.
- Tian, Z., Li, J., Jia, X., Yang, F., and Wang, Z. (2016). Assimilation and Translocation of Dry Matter and Phosphorus in Rice Genotypes Affected by Salt-Alkaline Stress. *Sustainability*, 8(6), 568.
- Tiwari, S., Krishnamurthy, S., Kumar, V., Singh, B., Rao, A., Rai, V., Singh, A. K., and Singh, N. K. (2016). Mapping QTLs for Salt Tolerance in Rice (*Oryza sativa* L.) by Bulked Segregant Analysis of Recombinant Inbred Lines Using 50K SNP Chip. *PloS one*, 11(4), e0153610.
- Trijatmiko, K. R., Prasetyono, J., Thomson, M. J., Cruz, C. M. V., Moeljopawiro, S., and Pereira, A. (2014). Meta-analysis of quantitative trait loci for grain yield and component traits under reproductive-stage drought stress in an upland rice population. *Molecular Breeding*, 34(2), 283-295.
- Tripathy, J., Zhang, J., Robin, S., Nguyen, T. T., and Nguyen, H. (2000). QTLs for cell-membrane stability mapped in rice (*Oryza sativa* L.) under drought stress. *Theoretical and Applied Genetics*, 100(8), 1197-1202.
- Tuan, V. D., Fukuta, Y., Tano, M., and Ban, T. (2000). Mapping quantitative trait loci for salinity tolerance in rice. *Omonrice*, 8(1), 27-35.
- Ulloa, M., Meredith Jr, W., Shappley, Z., and Kahler, A. (2002). RFLP genetic linkage maps from four F<sub>2:3</sub> populations and a joinmap of *Gossypium hirsutum* L. *Theoretical and Applied Genetics*, 104(2-3), 200-208.
- Valipour, M. (2014). Drainage, waterlogging, and salinity. *Archives of Agronomy and Soil Science*, 60(12), 1625-1640.
- Van Ooijen, J., and Kyazma, B. (2004). MapQTL® 5. *Software for the mapping of quantitative trait loci in experimental populations*. Kyazma BV, Wageningen. 111-169.
- Vega-Jarquín, C., García-Mendoza, M., Jablonowski, N., Luna-Guido, M., and Dendooven, L. (2003). Rapid immobilization of applied nitrogen in saline-alkaline soils. *Plant and Soil*, 256(2), 379-388.
- Venuprasad, R., Bool, M., Quiatchon, L., Cruz, M. S., Amante, M., and Atlin, G. (2012). A large-effect QTL for rice grain yield under upland drought stress on chromosome 1. *Molecular Breeding*, 30(1), 535-547.

- Vibhuti, C. S., Bargali, K., and Bargali, S. (2015). Seed germination and seedling growth parameters of rice (*Oryza sativa* L.) varieties as affected by salt and water stress. *Indian Journal of Agricultural Sciences*, 85(1), 102-108.
- Vikram, P., Swamy, B. M., Dixit, S., Trinidad, J., Cruz, M. T. S., Maturan, P. C., Amante, M., and Kumar, A. (2016). Linkages and Interactions Analysis of Major Effect Drought Grain Yield QTLs in Rice. *PloS one*, 11(3), 1-16.
- Vinocur, B., and Altman, A. (2005). Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology*, 16(2), 123-132.
- Vinod, K. K. (2006). Genome Mapping In Plant Populations. *Proceedings of training programme on "Modern approaches in Plant Genetics r esources-collection, conservation and utilization"*, Tamil Nadu Agricultu ral University, Coimbatore, India, 12pp.
- Wang, S., Basten, C., and Zeng, Z. (2007). Windows QTL cartographer 2.5. *Department of Statistics, North Carolina state university, Raleigh, NC*.
- Wang, W. S., Zhao, X. Q., Li, M., Huang, L. Y., Xu, J. L., Zhang, F., Cui, Y. R., Fu, B. Y., and Li, Z. K. (2015). Complex molecular mechanisms underlying seedling salt tolerance in rice revealed by comparative transcriptome and metabolomic profiling. *Journal of experimental botany*, erv476.
- Wang, Z., Cheng, J., Chen, Z., Huang, J., Bao, Y., Wang, J., and Zhang, H. (2012). Identification of QTLs with main, epistatic and QTL  $\times$  environment interaction effects for salt tolerance in rice seedlings under different salinity conditions. *Theoretical and Applied Genetics*, 125(4), 807-815.
- Wang, Z., Wang, J., Bao, Y., Wu, Y., and Zhang, H. (2011). Quantitative trait loci controlling rice seed germination under salt stress. *Euphytica*, 178(3), 297-307.
- Wissuwa, M., Yano, M., and Ae, N. (1998). Mapping of QTLs for phosphorus-deficiency tolerance in rice (*Oryza sativa* L.). *Theoretical and Applied Genetics*, 97(5-6), 777-783.
- Xia, J., Yamaji, N., Che, J., Shen, R. F., and Ma, J. F. (2014). Differential expression of *Nrat1* is responsible for Al-tolerance QTL on chromosome 2 in rice. *Journal of Experimental Botany*, 65(4), 297-304.
- Xu, D. (2012). Genetic studies on saline and sodic tolerances in soybean. *Breeding Science*, 61(5), 559.

- Xu, S. (2013). Mapping Segregation Distortion Loci. In *Principles of Statistical Genomics* Springer 151-170.
- Yadav, S., Irfan, M., Ahmad, A., and Hayat, S. (2011). Causes of salinity and plant manifestations to salt stress: a review. *Journal of Environmental Biology*, 32(5), 667.
- Yan, G. (2006). Germination Characteristics of Rice Seed under Salt Stress and Physiological Basis of Salt-tolerance of Rice in Seedlings. *Journal of Anhui Agricultural Sciences*, 34(6), 1053.
- Yan, W. H., Wang, P., Chen, H. X., Zhou, H. J., Li, Q. P., Wang, C. R., Ding, Z. H., Zhang, Y. S., Yu, S. B., and Xing, Y. Z. (2011). A major QTL, Ghd8, plays pleiotropic roles in regulating grain productivity, plant height, and heading date in rice. *Molecular Plant*, 4(2), 319-330.
- Yang, X., Romheld, V., and Marschner, H. (1994). Effect of bicarbonate on root growth and accumulation of organic acids in Zn-inefficient and Zn-efficient rice cultivars (*Oryza sativa* L.). *Plant and Soil*, 164(1), 1-7.
- Yao, M. Z., Wang, J. F., Chen, H. Y., Zhai, H. Q., and Zhang, H. S. (2005). Inheritance and QTL mapping of salt tolerance in rice. *Rice Sci*, 12(1), 25-32.
- Yeo, A. R., and Flowers, T. J. (1986). Salinity resistance in rice (*Oryza sativa* L.) and a pyramiding approach to breeding varieties for saline soils. *Functional Plant Biology*, 13(1), 161-173.
- Yeo, A., Yeo, M., Flowers, S., and Flowers, T. (1990). Screening of rice (*Oryza sativa* L.) genotypes for physiological characters contributing to salinity resistance, and their relationship to overall performance. *TAG Theoretical and Applied Genetics*, 79(3), 377-384.
- Yoshinaga, S., Takai, T., Arai-Sanoh, Y., Ishimaru, T., and Kondo, M. (2013). Varietal differences in sink production and grain-filling ability in recently developed high-yielding rice (*Oryza sativa* L.) varieties in Japan. *Field Crops Research*, 150(1), 74-82.
- Young, N. D. (1996). QTL mapping and quantitative disease resistance in plants. *Annual Review of Phytopathology*, 34(1), 479-501.
- Young, N. D. (2001). Constructing a plant genetic linkage map with DNA markers. In *DNA-Based Markers in Plants* (pp. 31-47): Springer.

- Zaare, M., and Jafary, H. (2013). Quantitative trait loci diversity for salt tolerance at the early growth stage of barley (*Hordeum vulgare* L.). *Crop Breeding Journal*, 3(2), 69-77.
- Zehra, A., Gul, B., Ansari, R., and Khan, M. (2012). Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. *South African Journal of Botany*, 78, 122-128.
- Zeng, L., and Shannon, M. C. (2000). Salinity effects on seedling growth and yield components of rice. *Crop Sci*, 40(1), 996-1003.
- Zeng, Z. B. (1994). Precision mapping of quantitative trait loci. *Genetics*, 136(4), 1457-1468.
- Zhang, G. Y., Guo, Y., Chen, S. L., and Chen, S. Y. (1995). RFLP tagging of a salt tolerance gene in rice. *Plant Science*, 110(2), 227-234.
- Zhang, J. T., and Mu, C. S. (2009). Effects of saline and alkaline stresses on the germination, growth, photosynthesis, ionic balance and anti-oxidant system in an alkali-tolerant leguminous forage *Lathyrus quinquenervius*. *Soil Science and Plant Nutrition*, 55(5), 685-697.
- Zhao, X., Daygon, V. D., McNally, K. L., Hamilton, R. S., Xie, F., Reinke, R. F., and Fitzgerald, M. A. (2016). Identification of stable QTLs causing chalk in rice grains in nine environments. *Theoretical and Applied Genetics*, 129(1), 141-153.
- Zhao, Y., Lu, Z., and He, L. (2014). Effects of saline-alkaline stress on seed germination and seedling growth of *Sorghum bicolor* (L.) Moench. *Applied Biochemistry and Biotechnology*, 173(7), 1680-1691.
- Zhiyuan, N., Zhao, R., Chen, H., Ai, N., Zhang, X., Zhao, J., Mei, H., Wang, P., Guo, W., and Zhang, T. (2013). Molecular tagging of a major quantitative trait locus for broad-spectrum resistance to *Verticillium* wilt in upland cotton cultivar Prema. *Crop Science*, 53(6), 2304-2312.
- Zhou, S., Bechner, M. C., Place, M., Churas, C. P., Pape, L., Leong, S. A., Runnheim, R., Forrest, D. K., Goldstein, S., and Livny, M. (2007). Validation of rice genome sequence by optical mapping. *BMC Genomics*, 8(1), 1.
- Zhou, W. C., Kolb, F. L., Bai, G. H., Domier, L. L., Boze, L. K., and Smith, N. J. (2003). Validation of a major QTL for scab resistance with SSR markers and use of marker assisted selection in wheat. *Plant Breeding*, 122(1), 40-46.

Zou, Z., Ishida, M., Li, F., Kakizaki, T., Suzuki, S., Kitashiba, H., and Nishio, T. (2013). QTL analysis using SNP markers developed by next-generation sequencing for identification of candidate genes controlling 4-methylthio-3-butenyl glucosinolate contents in roots of radish, *Raphanus sativus* L. *PLoS One*, 8(1), e53541.